

P011 Structure-based mechanism of CMP-Kdo synthetase: unusual Kdo-dependent activation of the CTP α -phosphate to act as general base

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The enzyme CMP-Kdo synthetase (KdsB) catalyzes the addition of 2-keto-3-deoxy-*manno*-octulonic acid (Kdo) to CTP to form CMP-Kdo, which is a key reaction in the biosynthesis of lipopolysaccharide. The reaction catalyzed by the bacterial KdsB and the related eukaryotic enzyme CMP-acylneuraminate synthase (CNS) is unique among the sugar-activating enzymes in that the respective sugars are directly coupled to a cytosine monophosphate (as opposed to using a phosphorylated sugar to produce a sugar nucleotide diphosphate). We here present a new continuous steady-state assay for the reaction that allows a detailed kinetic characterization of the lipopolysaccharide specific isozyme of the *E. coli* enzyme. The K_m for Kdo is shown to be $\sim 2\mu\text{M}$ and the K_m for CTP $4.8 \pm 0.5 \mu\text{M}$ with a k_{cat} value of $3.8 \pm 0.02 \text{ s}^{-1}$. Subsequent inhibition studies, in combination with isothermal titration calorimetry binding assays, show the substrate analogue 2 β -deoxy Kdo to be a potent competitive inhibitor of the enzyme. The 2.5 Å ligand-free KdsB and the 1.8 Å ternary complex KdsB:CTP:2 β -deoxy-Kdo crystal structures reveal that Kdo binding leads to active site closure with concomitant repositioning of the CTP phosphate moieties and associated Mg^{2+} ion. This leads to the unusual Kdo-dependent activation of the α -phosphate to act as general base for deprotonation of the Kdo substrate. We propose the related CNS enzymes to use a similar mechanism leading to the formation of CMP-5-N-acetylneuraminic acid, one of the most abundant sialic acids.